The unamplified SGBAF-1 cDNA library (10^6 recombinants) was plated on E.coli K12 PLK-F' (Stratagene) at a density of 10^5 plaques per 15 cm dish and lifts taken in duplicate onto nitrocellulose membranes (Millipore). For screening, filters were prehybridized for at least 1 h at 42°C in 6 x SSPE, 0.5% SDS, 10 x Denhardt's solution, 100 μg ml^{-1} denatured sonicated herring sperm DNA (Sigma). Hybridization was carried out in the same solution containing 10 ng ml^{-1} radiolabelled oligonucleotide. Oligonucleotides used were: Peptide N (MDWIFHT) (SEQ ID NO: 11) 5'-AA(G/A)ATTGGA(T/C)TGGAT(C/T/A)TT(T/C)CA(T/C)AC-3') (SEQ ID NO: 12); Peptide J(D D G Q L F H I D F G H F) (SEQ ID NO: 13); 5'-GATGATGGCCA(G/A)CTGTT(T/C)CA(T/C)AT(T/A)GA(T/C)TTTGGCCA(T/C)T T (SEQ ID NO: 14). Oligonucleotides were labelled with ^{32}P at the 5' end in a 20 μl reaction containing 100 ng oligonucleotide, 1 x kinase buffer (Promega), 0.1 mM spermidine, 5 mM dithiothreitol, 100 μCi [γ-^{32}P]ATP (5000 Ci $mmol^{-1}$, Amersham) and 2 μl (200 U) 54 polynucleotide kinase (Amersham). Filters were washed in 6xSSC, 0.1% SDS at room temperature and then subjected to autoradiography using Kodak XAR film. Hybridizing clones were plaque-purified and rescued as plasmids according to the manufacturing instructions.

F1

Page 39, line 18-page 40, line 6: replace by the following:

RACE PCR was carried out essentially as published previously (Frohman, et al., 1988; Harvey and Garlison, 1991). Briefly, first strand cDNA primed with random hexamers (Amersham) was synthesized from 1 μg of SGBAF-1 cell mRNA using the Stratagene first strand cDNA synthesis kit. First strand cDNA was isolated by isopropanol precipitation and tailed with oligo-dA using terminal deoxynucleotidyl transferase (BRL). PCR was performed using oligo 2224 (5'-AATTCACACACTGGCATGCCGAT) (SEQ ID NO: 15) and adaptor-dT (5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTT) (SEQ ID NO: 16) as primers using a Perkin Elmer/Cetus Tap polymerase PCR kit (conditions: 94°C 1 min, 35°C 1 min, 72°C 2 min, 30 cycles). Products were fractionated on a 1.5% low melting point agarose gel and visualized by staining with ethidium bromide. The gel was sliced into 6 bands (size range 150-2000 bp) and DNA isolated from each gel slice. A further round of PCR 2 was performed on this DNA using oligonucleotide 2280 (5' –TTTAAGCTTAGGCATTCTAAAGTCACTATCATCCC) (SEQ ID NO: 17) and adaptor (5'-GACTCGAGTCGACATCGA) (SEQ ID NO: 18) as primers (conditions: 94°C 1 min, 56 °C 1 min, 72°C min, 356 cycles. Products were fractioned on an agarose gel and visualized by staining with ethidium bromide. A band 250 bp shorter than the size of the DNA in the gel slice used for the PCR was expected. An intensely staining band of 350 bp obtained from the ~600 bp gel slice was excised, digested with Hind111 and Sall and ligated into Bluescript KS- digested with Hind111 and Xhol to give plasmid pBS/race. Two independent inserts were completely sequenced.

F2

Page 41, lines 12-35: replace by the following:

For p85α 125 ng of poly $(A)^+$ RNA was reverse transcribed with 2.5 units rTth DNA polymerase (Perkin-Elmer-Cetus) at 70°C for 10 min in a 10 µl reaction containing 10 mM Tris-HCl (pH 8.3), 90 mM KCl, 1mM MnCl, 0.5 mM dNPT mixture and 1.2 µM antisense primer (5'-CAGGCCTGGCTTCCTGT) (SEQ ID NO: 19). For DNA polymerization the reaction volume was adjusted to 50 µl by adding a single mix giving the following final concentrations: 5% (v/v) glycerol, 10 mM Tris-HCl (pH 8.3), 100 mM KCl, 0.75 mM EGTA, 0.05% (v/v) Tween 20, 2 mM $MgCl_2$, 0.24 µM sense primer (5'-AACCAGGCTCAACTGTT) (SEQ ID NO: 20). PCR was then performed under the following reaction conditions: 92°C 1 min, 58°C 1 min, 72°C 1 min for 25 cycles on a Perkin Elmer-Cetus DNA thermal cycler.

F3

Conditions for p110 were similar except concentration of the antisense primer (5'-TGCTGTAAATTCTAAATGCTG) (SEQ ID NO: 21) was increased to 4.8 µM during the reverse transcription step. DNA polymerisation conditions were the same except the final $MgCl_2$ concentration was increased to 2.5 mM and both primers (sense primer = 5'-GTATTTCATGAAACAAATGA) (SEQ ID NO: 22) were present at a final concentration of 0.96 µM. Taq DNA ploymerase (Promega) was also added at 0.03 U μl^{-1} . PCR was performed as follows: 92°C 30 sec, 54°C 5 sec, 72°C 30 sec for 35 cycles. 20 µl of each reaction was run on a 3% agarose gel (Maniatis, et al. 1982) and visualised by staining with ethidium bromide.

Page 41, line 36-page 42, line 10: replace by the following:

For the preparation of the anti C-terminal p110 antiserum, peptide CKMDWIFHTIKQHALN was synthesized by FMOC chemistry and purified by HPLC. It was then coupled to KLH using glutaraldehyde, and injected into the lymph nodes of rabbits using methods described in Kypta, R M et al., (1990), Cell 62, 481-492. Positive antisera as determined by enzyme-linked immunoassay were affinity purified on specific peptide-Actigel affinity columns. Anti-p85α (Otsu, et al., 1991) and anti CSF-1 receptor (Ashmun, et al., 1989) antisera are previously documented. Immunoprecipitations were carried out as described in Otsu, et al., 1991.

F4

Page 52, line 26-page 53, line 4: replace by the following:

The human cDNA was isolated from a cDNA library, made from mRNA isolated from the human cell line KG1a using standard techniques. The probe was a partial cDNA from the second half of the bovine p110 cDNA. The probe was labelled with ^{32}P and hybridised overnight to the library filters at 65°C in 1M NaPi, 7% SDS buffer. The filters were washed in 2xSSC at 50°C, and exposed to X-ray film at -70°C. The nucleotide sequence is shown in Figure 16 together with the corresponding amino acid sequence. The human p110 sequence has 95% homology to the bovine p119 sequence at the DNA level and is 98% identical at the protein level (Figures 17 and 18). The protein sequence is shown in

F5

Figure 19. Primers (357) AAG GAT CAG AAC AAT GCC T (SEQ ID NO: 24) and (416) AGG CTT TCT TTA GCC ATC A (SEQ ID NO: 25) were used to amplify, using RT-PCR (94°C 30 sec 50°C sec, 72°C 60 secs; for 35 cycles) the partial sequence of a highly related p110 gene (p110-11). P110-11 has 96% nucleotide homology to p110 (sequence not provided).

Page 53, lines 5-23: replace by the following:

Two novel cDNAs related to p110 have been cloned. Degenerate primers were designed to conserved sequences between human p110 and the related yeast gene VPS34 (Sense (GDDLRQD) (SEQ ID NO: 26) 5' GGN GAT/C GAT/C T/C TA/G CGN CAA/G GA-3 (SEQ ID NO: 27) antisense (FHIDFGHF) (SEQ ID NO: 28) 5' A/GAA A/GTG ICC A/GAA A/GTC A/G/TAT A/GTG A/GAA-3) (SEQ ID NO: 29). These were used in RT-PCR reactions using mRNA from the human cell lines MOLT4 and U937 (94°C 30 sec, 50°C 30 sec, 74°C 30 sec for 35 cycles). Two novel cDNA's, PITR-c and PITR-f, related to p110, were isolated. The PITR-c nucleotide sequence is shown in Figure 20. This gene is highly related to the yeast gene VPS34, the VPS34 protein is involved in the protein sorting from the golgi to the vacuole and has an intrinsic PI3-kinase activity. The PITR-f nucleotide sequence is shown in Figure 21 and is more similar to p119 than PITR-c and is likely also to possess PI3-kinase activity. The alignment of human p110, the human PI3-kinase related genes PITR-c and PITR-f and the yeast PI3-kinase VPS34 are shown in Figure 22. The amino acids conserved in 3 or more of the proteins are shown in the upper case.

IN THE CLAIMS

Cancel claims 39-50 without prejudice.

Add claims 51-61 which follow:

Claim 51: A method for determining expression of a gene which encodes a human polypeptide that has PI3 kinase activity and a molecular weight of about 110 kiladaltons as determined by SDS-PAGE, comprising contacting a sample with a nucleic acid molecule which hybridizes specifically to a transcript of said gene, at 1MNaCl, 10xDenhardt's solutions; 50mM Tris-HCL (pH 7.4); 10mM EDTA; 0.1%SDS; 100μg/ml denatured herring sperm DNA at 65°C for 16 hours, followed by a wash of 2XSSC; 0.1%SDS at 42°C, or a wash of 0.5XSSC/0.1% SDS at 50°C, or a wash at 0.1XSSC/0.1%SDS at 65°C, or a wash at 0.1XSSC/0.1% SDS, at 68°C and determining said hybridization as a determination of expression of said gene.

Claim 52: The method of claim 51, wherein said nucleic acid molecule is labeled with ^{32}P.